Functioning of a metabolic flux sensor in Escherichia coli

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Supporting Information

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SI Text 1: Model Describing System Without Feedforward Activation

The mathematical model containing two metabolites (X and Y) and two enzymatic reactions (E_1 and E_2) can be described by a set of two ordinary differential equations:

$$\frac{dX}{dt} = v - v_{E1} \quad \textbf{[S1]}$$

$$\frac{dY}{dt} = v_{E1} - v_{E2}, \quad \textbf{[S2]}$$

where v denotes the influx (input of the system) and v_{E1} and v_{E2} denote the reaction rates of E_1 and E_2, respectively. At steady state, the differential expressions are zero, simplifying Eqs. **S1** and **S2** to the following:

$$v_{E1} = v_{E2} = v. \quad \textbf{[S3]}$$

The rates through the two reactions v_{E1} and v_{E2} can be described with the following equations, assuming a reversible Michaelis–Menten-type kinetic for E_1 and an irreversible Michaelis–Menten-type kinetic for E_2:

$$v_{E1} = v = \frac{v_{\max.E1} \cdot \left(X - \frac{Y}{K_{eq}}\right)}{K_{m.X.E1} \cdot \left(1 + \frac{Y}{K_{m.Y.E1}}\right) + X} \quad \textbf{[S4]}$$

$$v_{E2} = v = \frac{v_{\max.E2} \cdot Y}{K_{m.Y.E2} + Y}. \quad \textbf{[S5]}$$

$K_{m.X.E1}$, $K_{m.Y.E1}$, and $K_{m.Y.E2}$ denote the K_m values for X and Y of E_1 and for Y of E_2, respectively. K_{eq} denotes the equilibrium constant of E_1, and $v_{\max.E1}$ and $v_{\max.E2}$ denote the maximal possible fluxes of E_1 and E_2, respectively.

To obtain an analytical solution of the relationship of X and the flux v, Eqs. **S4** and **S5** can be rearranged for X and Y, respectively. Replacing Y in Eq. **S4** (and assuming that $K_{m.X.E1} = K_{m.Y.E1}$ to reduce the number of parameters), one obtains the following:

$$X = \frac{v \cdot K_{m.X.E1}}{v_{\max.E1} - v} + \frac{v \cdot K_{m.Y.E2} \cdot v_{\max.E1}}{K_{eq} \cdot (v_{\max.E1} - v) \cdot (v_{\max.E2} - v)} + \frac{v^2 \cdot K_{m.Y.E2}}{(v_{\max.E1} - v) \cdot (v_{\max.E2} - v)}. \quad \textbf{[S6]}$$

For $v << v_{\max.E1}$ and $v << v_{\max.E2}$ (which is equivalent to an influx that is much lower than the maximal possible fluxes for E_1 and E_2), this equation simplifies to the following:

$$X = \frac{v \cdot K_{m.X.E1}}{v_{\max.E1}} + \frac{v \cdot K_{m.Y.E2}}{K_{eq} \cdot v_{\max.E2}}. \quad \textbf{[S7]}$$

This equation describes the analytical solution for the relationship of X and flux v.

SI Text 2: Model Describing System with Feedforward Activation of E_2 by X

Here, we use the same ordinary differential equations as above. In contrast to the previous model, we use a Monod–Wyman–Changeux (MWC) kinetic for E_2 in accordance to previous studies on pyruvate kinase I (PYK I) (1–3):

$$v_{E2} = v = \frac{v_{\max.E2} \cdot \frac{Y}{K_{m.Y.E2}} \cdot \left(1 + \frac{Y}{K_{m.Y.E2}}\right)^{n-1}}{L \cdot \left(1 + \frac{X}{K_{mA.X.E2}}\right)^{-n} + \left(1 + \frac{Y}{K_{m.Y.E2}}\right)^{n}}. \quad \textbf{[S8]}$$

L, n, and $K_{mA.X.E2}$ in Eq. **S8** denote allosteric equilibrium constant, cooperativity constant, and affinity constant of X for E_2, respectively. We chose values for L and n in accordance to parameter values that were obtained for PYK I in previous studies: PYK I is inactive in absence of its allosteric activator FBP (1, 4), which corresponds to $L >> 1$, and several studies have determined n to be equal to 4 (1, 5). Because $n > 1$, it is not possible to derive an analytical solution for the relationship of X and flux v, and thus we solve this model equation as specified in the main text.

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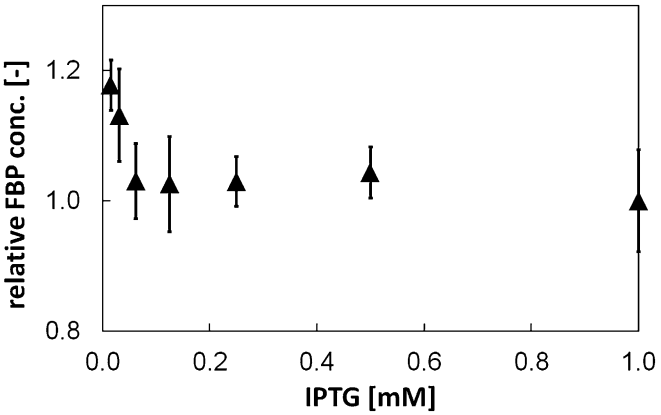

Fig. S1. Abundance of intracellular FBP as a function of IPTG concentration (used as a proxy for *pykF* abundance) in glucose batch cultures of a *pykF* mutant strain bearing an IPTG-inducible PYK I expression plasmid, relative to the FBP concentration at 1 mM IPTG. Batch cultivations were performed in 500-mL shake flasks containing 30 mL of M9 glucose medium (5 g/L glucose) as described in the main text. Cultures were harvested in midexponential growth phase using fast filtration and extracted in hot ethanol as described previously (1). Extracts were then analyzed by LC-MS/MS as described in the main text.

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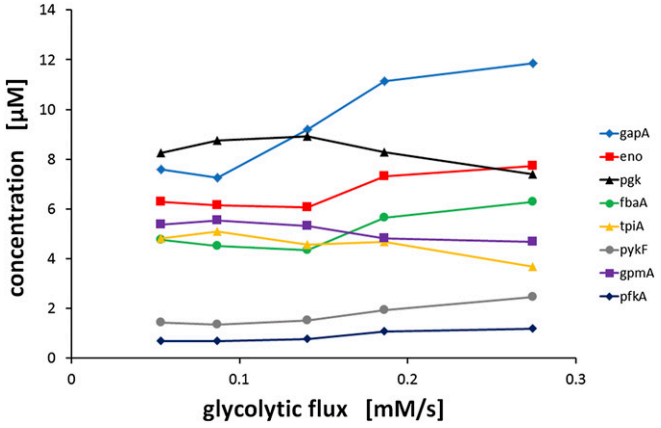

Fig. S2. Intracellular concentrations of enzymes of lower glycolysis at different glycolytic fluxes. Whereas the data from the four lower flux values stem from glucose-limited chemostat cultures, the highest flux data point was obtained from a glucose batch culture. Enzyme copy numbers were determined by targeted MS analysis and heavy reference peptides (1) and then converted to concentrations using the cell volumes measured in ref. 2.

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